



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Robert C. Ladner *et al.* Art Unit : 1639
Serial No. : 10/656,350 Examiner : Jeffrey S. Lundgren
Filed : September 5, 2003
Title : DISPLAY LIBRARY PROCESS

Commissioner for Patents
Washington, D.C. 20231

DECLARATION UNDER 37 CFR 1.131

I, Kristin Rookey, a citizen of the United States, residing at 14 Hancock Street, Revere, MA 02151, U.S.A., hereby declare as follows:

1. I am an inventor of the subject matter disclosed and claimed in the above-referenced United States Patent Application.
2. I am familiar with the present claims of the application, which are directed to, *inter alia*, methods of selecting phage that encode a target binding protein from a plurality of display phage.
3. Prior to June 21, 2002, I had reduced my invention to practice as described and claimed in the above-identified application in this country, a NAFTA country or WTO country, as evidenced below.
4. I submit herewith Exhibits B-D evidence showing reduction to practice of the claimed invention prior to the June 21, 2002. The date on each page is redacted. Each page is dated prior to June 21, 2002.

Exhibit B is an excerpt from my laboratory notebook showing experiments that included three rounds of phage selection.

Exhibit C is an entry from my laboratory notebook with experiments showing multiple rounds of phage selection, followed by tests of specificity of selected positives by ELISA.

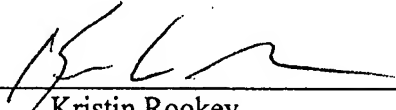
Exhibit D is an entry from my laboratory notebooks showing the diversity of selected positive clones (as seen on the agarose gels).

5. In sum, I submit herewith evidence (Exhibits B-D) that shows reduction to practice of the claimed methods prior to June 21, 2002.

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6. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment, under Title 18 § 1001 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

5-4-07
Date


Kristin Rookey

CONTINUED FROM

Object: Test out a new method of selections whereby all 3 rounds of selections are done in 1 day.

1. block 100ml Dynal M250 SA magnetic beads w/ 2% MPBS - total vol = 500ml (3 separate runs of 100ml each)
2. SA phase

$$K = 1.5 \times 10^6 \text{ per ml}$$

$$L = 1.5 \times 10^6 \text{ per ml}$$

10ml of each (3x10" total) to blocked SA beads. Rinse vol to 500ml w/ 2% MPBS
60 min RT

3. WASH 3x w/ PBS + .01% tween
4. add 500ml of XL1 Blue MRF' cells OD₆₀₀ = .455 transfer to 50ml conical

#1 37°C 20min
30°C 25min

#2 37°C 45min

#3 30°C 45min

AT 15 min add 5ml of 100mM IPTG to get 1mM IPTG

5. Remove bacteria, Titer on XL41 & XL2 10ml 1:1:0.01ml
6. WASH 3x PBS + .01% tween
7. Repeat steps 4-6 2 more times
8. expand the 500ml XL1 Blue MRF' outprint cultures to 10ml 2xYT + 1mM IPTG 30°C o/n

Count colonies & pfu

37°C/30°C

Round #1

1168 on .01

$5.84 \times 10^7 \text{ pfu}$

input = 3×10^{11}

eff = 1.95×10^{-4}

WILLIAM AND GERTIE

Edward H. Cohen

[Signature]

CONTINUED FROM

37°C Round 2

101 on .1 ml

 5.1×10^5 cfuinput = 5.8×10^7 cfufoi = 8.8×10^{-3} 12ms
each)

Round 3 146 on 1 ml

 7.3×10^4 cfufoi = 1.4×10^{-1} 37°C 45 min

Round 1 1602 on .01 ml

 8×10^7 cfufoi = 2.7×10^{-4}

Round 2 241 on .01 ml

 1.2×10^6 cfufoi = 6.5×10^{-2}

Round 3 107 on 1 ml

 5.4×10^4 cfufoi = 4.5×10^{-2}

1 ml DPT

30°C 45 min

.1 ml .01 ml

Round 1 1076 on .01 ml

 5.13×10^7 cfufoi = 1.7×10^{-4}

Round 2 1500 on 1 ml

 7.6×10^5 cfufoi = 1.2×10^{-2}

Round 3 95 on 1 ml

 4.75×10^4 cfufoi = 6.3×10^{-2}

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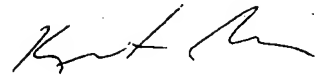
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1613-038

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SUBJECT

CONTINUED FROM

pfu

37°/30°C

Round 1 5×10^8 pfu
 2 2×10^7 pfu $f_{DI} = 4 \times 10^{-2}$
 3 4.3×10^6 pfu $f_{DI} = 2.1 \times 10^{-1}$

39°C

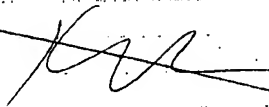
Round 1 5×10^8 pfu
 2 2.6×10^7 pfu $f_{DI} = 5.2 \times 10^{-2}$
 3 2.1×10^6 pfu $f_{DI} = 7.8 \times 10^{-2}$

30°C

Round 1 5×10^8 pfu
 2 2.5×10^7 pfu $f_{DI} = 5 \times 10^{-2}$
 3 3.35×10^6 pfu $f_{DI} = 1.3 \times 10^{-1}$

- NOTE - enrichment is positive between rounds
 - attempt additional targets
~~- fingerprint on the future PCR~~
 - pick colonies & attempt assays

24hr + 1hr DDTB 30°C in 96 well plate



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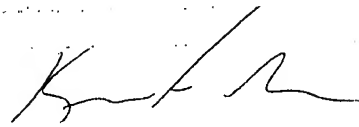
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CONTINUED FROM

Object: 2nd attempt at Fast Screen at Fick on R heads

1. block 100 ml of Sybral mag 20's SA magnetic beads
22 MBBS 60 min
(rinse w/ MBBS 2x prior to blocking)

2. 10 ml of K (1.5×10^{10} /ml)
10 ml of L (1.5×10^{10} /ml)

1.43 ml Fick BSA .7% / ml

Raise vol to 50 ml in 22 MBBS

60 min on rotator

3. Remove block from beads

4. add 50 ml of Fick BSA / phage to beads
Raise vol to 50 ml w/ 22 MBBS
30 min on rotator

5. WASH beads 5x PBS + .01% Tween

6. add 500 ml KL75/ml MBBS $OD_{600} = .5$

- Move to 50 ml CORICAL

37°C 20 min

5 15 min Add 5 ml 100 mM IPTG

30°C 25 min

7. Remove bacteria

Take 100 ml 10⁻¹ - 2 - 3 on BL41

spread bacteria to 10 ml culture UYT + 1 mM IPTG

30°C O/N

8. WASH beads 3x PBS + .01% Tween

Repeat 6-7 2 more times

Count Cfu

R1 10⁻³ = 800 Cfu

R2 10⁻³ = 156

R3 10⁻³ = 25

total Cfu

4×10^6

7.8×10^5

1.25×10^5

BAE

1.3×10^{-5}

2×10^{-1}

1.6×10^{-1}

- expand colonies for ELISAs

30°C O/N in UYT + 1 mM IPTG 96 well plate

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1613-040

DATE

SUBJECT

OueAs - SA 4672

CONTINUED FROM

object: test Fast Screen phase output for Specificity

1. Cont. of Immunol. 2. 100mg Gr. BA / 100ml. ^{100mm} Sod. Bicarb. pH 8.5
 6x " " 100mg SA / 100ml " "

0.1N HCl

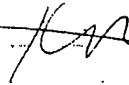
2. block 1 hour 20 M PBS 100ml/well
3. WASH 3x 100ml 0.05% Tween
4. add 100ml Supersatant each well on SA & on Ritz
 60min RT
5. WASH
6. 100ml/well 1M13-NRP Amersham 15000 60min
7. WASH
8. TRIS

pick 12 Fite positives from R3 to continue with
 B9 D8 D10 E3 E9 F9 F10 G1 G10 H1 H4 H10

pick 9 SA positives from R2

MA MB C3 C10 E5 E11 F4 F8 F10

See page 42



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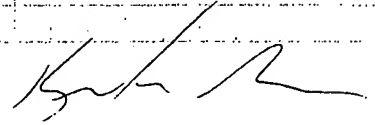
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1613-042

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SUBJECT

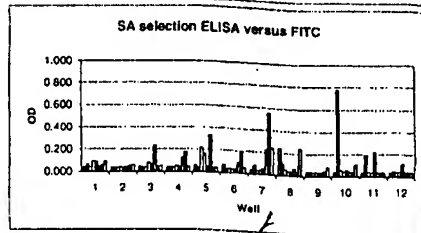
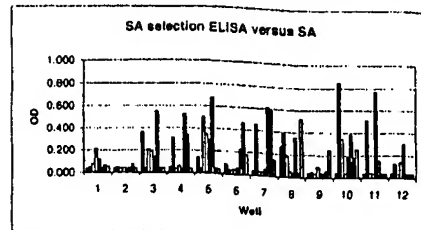
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SA on SA

0.035	0.039	0.368	0.011	0.140	0.068	0.038	0.218	0.043	0.043	0.042	0.043
0.050	0.053	0.039	0.310	0.038	0.037	0.457	0.384	0.051	0.848	0.511	0.127
0.078	0.039	0.208	0.035	0.504	0.056	0.036	0.187	0.038	0.343	0.048	0.037
0.214	0.047	0.194	0.060	0.345	0.079	0.037	0.051	0.096	0.040	0.039	0.147
0.126	0.037	0.142	0.036	0.295	0.052	0.059	0.044	0.045	0.185	0.769	0.305
0.042	0.040	0.550	0.526	0.682	0.220	0.613	0.353	0.030	0.360	0.154	0.038
0.070	0.077	0.048	0.340	0.040	0.464	0.597	0.044	0.058	0.180	0.038	0.035
0.056	0.042	0.041	0.037	0.040	0.180	0.145	0.522	0.245	0.252	0.038	0.040

SA on FITC

0.040	0.037	0.044	0.038	0.058	0.071	0.039	0.245	0.040	0.038	0.039	0.039
0.065	0.037	0.037	0.044	0.039	0.037	0.078	0.105	0.046	0.776	0.195	0.051
0.042	0.036	0.039	0.036	0.217	0.038	0.037	0.057	0.039	0.061	0.044	0.041
0.094	0.040	0.060	0.057	0.188	0.038	0.038	0.041	0.042	0.043	0.040	0.048
0.085	0.036	0.067	0.043	0.047	0.040	0.053	0.030	0.038	0.059	0.222	0.117
0.044	0.041	0.233	0.127	0.332	0.098	0.228	0.064	0.039	0.044	0.042	0.045
0.080	0.053	0.047	0.178	0.044	0.190	0.557	0.042	0.047	0.040	0.037	0.040
0.092	0.058	0.049	0.043	0.043	0.057	0.245	0.248	0.085	0.109	0.039	0.038

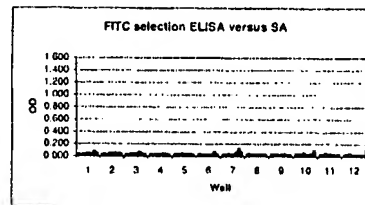
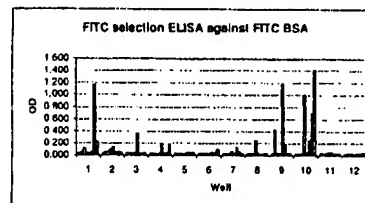


Fitc on Fitc

0.041	0.041	0.043	0.042	0.042	0.044	0.043	0.039	0.041	0.040	0.054	0.039
0.060	0.065	0.040	0.041	0.044	0.058	0.039	0.039	0.041	0.044	0.036	0.045
0.118	0.054	0.045	0.037	0.039	0.039	0.039	0.041	0.044	0.036	0.045	0.042
0.042	0.099	0.045	0.040	0.044	0.039	0.074	0.249	0.040	1.010	0.061	0.034
0.041	0.138	0.363	0.194	0.042	0.042	0.043	0.039	1.179	0.051	0.068	0.034
0.064	0.055	0.039	0.042	0.058	0.059	0.137	0.042	0.192	0.249	0.040	0.037
1.165	0.050	0.039	0.053	0.050	0.053	0.072	0.039	0.048	0.714	0.042	0.035
0.223	0.054	0.042	0.188	0.050	0.108	0.038	0.046	0.043	1.410	0.044	0.039

Fitc on SA

0.040	0.038	0.038	0.038	0.043	0.043	0.041	0.040	0.051	0.055	0.044	0.041
0.037	0.042	0.037	0.036	0.039	0.042	0.041	0.047	0.049	0.046	0.043	0.046
0.040	0.040	0.050	0.044	0.039	0.037	0.049	0.038	0.043	0.042	0.058	0.055
0.050	0.081	0.048	0.038	0.053	0.045	0.042	0.041	0.037	0.068	0.048	0.036
0.056	0.064	0.055	0.048	0.048	0.042	0.038	0.042	0.044	0.041	0.048	0.040
0.044	0.065	0.078	0.056	0.049	0.043	0.058	0.042	0.054	0.041	0.044	0.037
0.081	0.055	0.051	0.045	0.044	0.061	0.138	0.048	0.030	0.046	0.036	0.036
0.058	0.052	0.043	0.042	0.039	0.045	0.078	0.044	0.038	0.101	0.040	0.040



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SUBJECT

Fingerprint Ph/SA binders 1613-041

CONTINUED FROM

Object - fingerprint 12 vtr pressures & 9 SA pressures of
BSTNI

1. Colony PCR

10X PCR buffer II

10

95°C 5m

10 mM dNTPs

8

95°C 30s

25 mM MgCl₂

8

60°C 30s

ONT 166 Hind III 100 pmole

0.1

72°C 1m 30s

ONT 166 APL III 100 pmole

.1

72°C 5m

Taq

0.5

DNA

71.5

100µl

28x

2. Digest w/ BSTNI

10X BSA

2 ml

18X NEB2

0.5 ml

BSTNI 100 U/ml

1 ml

PCR prod

20 ml

25 ml

60°C

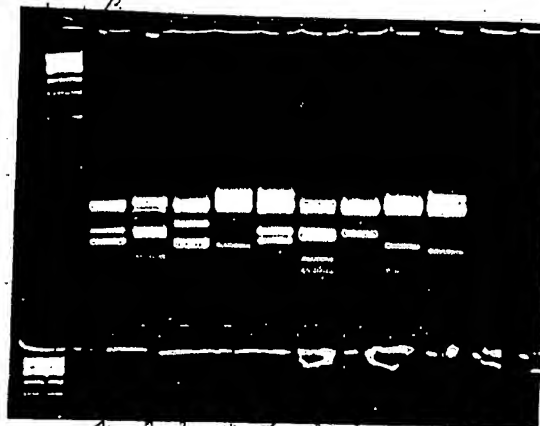
1 hour

3. 38 Agarose gel

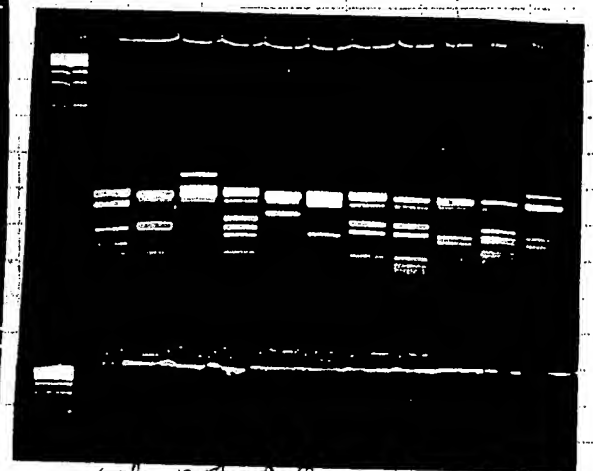
-load all

SA:

Ftr:



BT PB B3 B4 B5 B6 B7 B8 B9 B10



BT PB B3 B4 B5 B6 B7 B8 B9 B10 H1 H4

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